PATENT

**DOCKET NO.:** RUBC-0025 (97-0081 US)

**Application No.:** 09/763,331

Office Action Dated: January 15, 2003

This listing of claims will replace all prior versions, and listings, of claims in the application.

## **Listing of Claims:**

1. (Currently Amended) A synthetic fatty acid desaturase gene for expression in a multicellular plant, the gene comprising a desaturase domain of a fatty acid  $\Delta$ -9 desaturase and a cyt  $b_5$  domain, wherein the gene is customized for expression in a plant cytoplasm.

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- 2. (Currently Amended) The synthetic gene of claim 1, customized from a naturally occurring gene encoding a cytosolic Δ-9 desaturase and cyt b<sub>5</sub> domains wherein the desaturase domain is part of a dual domain desaturase/Cyt b<sub>5</sub> protein obtained from an organism selected from the group consisting of Saccharomyces cerevisiae, Pichia angusta, Histoplasma capsulatum, Cryptococcus curvatus, Yarrow lipolytica, and Kluyveromyces thermotolerans; and the cyt b5 domain is a cytochrome b<sub>5</sub> domain from an organism selected from the group consisting of Saccharomyces cerevisiae, Pichia angusta, Histoplasma capsulatum, Cryptococcus curvatus, Yarrow lipolytica, Kluyveromyces thermotolerans, Nicotiana tabacum, Oryza sativa, Cuscuta reflexa, Arabidopsis thaliana, Brassica oleracea, Olea europaea and Borago officinalis.
- 3. (Currently Amended) The synthetic gene of claim 1, customized from a naturally occurring cytosolic  $\Delta$ -9 desaturase gene from Saccharomyces cerevisiae.
- 4. (Currently Amended) The synthetic gene of claim 3, customized from a naturally occurring cytosolic  $\Delta$ -9 desaturase gene from Saccharomyces cerevisiae that encodes SEQ ID NO:2.

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5. (Currently Amended) The synthetic gene of claim 4, customized from a naturally occurring cytosolic Δ-9 desaturase gene from Saccharomyces cerevisiae comprising SEQ ID NO:1.

- 6. (Original) The synthetic gene of claim 3, comprising SEQ ID NO:3.
- 7. (Original) The synthetic gene of claim 1, which further comprises an expression regulatory sequence from a plant gene encoding an ER biosynthetic pathway enzyme.
- 8. (Original) The synthetic gene of claim 1, customized for expression in a monocotyledonous plant.
- 9. (Original) The synthetic gene of claim 1, customized for expression in a dicotyledonous plant.
- 10. (Original) The synthetic gene of claim 1, customized for expression in a plant genus selected from the group consisting of *Arabidopsis*, *Brassica*, *Phaeseolus*, *Oryza*, *Olea*, *Elaeis* (Oil Palm) and *Zea*.
- 11. (Original) The synthetic gene of claim 1, customized from a naturally occurring gene comprising both a desaturase domain and a cyt b<sub>5</sub> domain.

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12. (Original) The synthetic gene of claim 1, wherein the gene is a chimeric gene comprising a desaturase domain and a heterologous cyt b<sub>5</sub> domain.

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13. (Original) The synthetic gene of claim 1, customized from a naturally occurring gene such that the synthetic gene and the naturally occurring gene encode an identical amino acid sequence.

14. (Original) The synthetic gene of claim 13, wherein the synthetic gene and the naturally occurring gene encode SEQ ID NO:2.

15. (Original) The synthetic gene of claim 1, customized from a naturally occurring gene such that the synthetic gene and the naturally occurring gene encode a similar amino acid sequence.

16. (Original) The synthetic gene of claim 1, customized from a naturally occurring gene such that the synthetic gene and the naturally occurring gene encode a similar amino acid sequence, and the synthetic gene possesses improved stability or catalytic activity as compared with the naturally occurring gene.

17. (Currently Amended) A method for constructing a customized bifunctional fatty acid  $\Delta$ -9 desaturase/cyt  $b_5$  encoding gene for expression in the cytosol of a multicellular plant, comprising the steps of :

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(a) providing a DNA molecule comprising a <u>fatty acid  $\Delta$ -9</u> desaturase-encoding moiety operably-linked to a cyt b<sub>5</sub>-encoding moiety, said DNA molecule producing the bifunctional polypeptide in a non-customized form;

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(b) back-translating the polypeptide sequence using preferred codons for expression in a multicellular plant, thereby producing a back-translated nucleotide sequence;

(c) analyzing the back-translated nucleotide sequence for features that could diminish or prevent expression in the plant cytoplasm;

(d) modifying the analyzed sequence to correct or remove the features that could diminish or prevent expression in the plant cytoplasm; and

(e) optionally, introducing pre-determined cloning features into the sequence in a manner that does not materially affect the codon usage or final polypeptide sequence, thereby producing the customized bifunctional fatty acid  $\Delta$ -9 desaturase/cyt b<sub>5</sub> encoding gene for expression in the cytosol of a multicellular plant.

18. (Original) The method of claim 17, wherein the features that could diminish or prevent expression in the plant cytoplasm include one or more features selected from the group consisting of: putative intron splice sites, plant polyadenylation signals, RNA polymerase II termination sequences, and hairpin consensus sequence.

19. (Original) The method of claim 17, which further comprises the step of:

(f) testing the customized bifunctional desaturase/cyt b<sub>5</sub> encoding gene for desaturase function in fatty acid deficient strains of a microorganism prior introducing the genes into vectors for expression in plants.

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20. (Original) The method of claim 19, wherein the microorganism is Saccharomyces cerevisiae.

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21. (Cancelled)